

Supplemental Figure 1

Mitochondrial dynamics in cells of green cotyledons at 4-days post-germination.

(A) Representative image generated using Imaris to track mitochondria in cotyledon cells 4 days after germination. Detected mitochondria were colored green and tracks are rainbow colored according to speed (no movement is purple and movement above $0.310 \mu\text{m/s}$ is red). Bar = $5 \mu\text{m}$.

(B) Violin box plots of the mean speed recorded per track in nm/s. Box plot whiskers indicate $1.5 \times \text{IQR}$ and any outliers are represented by an empty circle, as described by Tukey. Means are represented by a full circle. The notch corresponds to the median $\pm 1.58 \times \text{IQR}/\sqrt{n}$. Total number of objects tracked was 550 per time point from 14 seedlings.

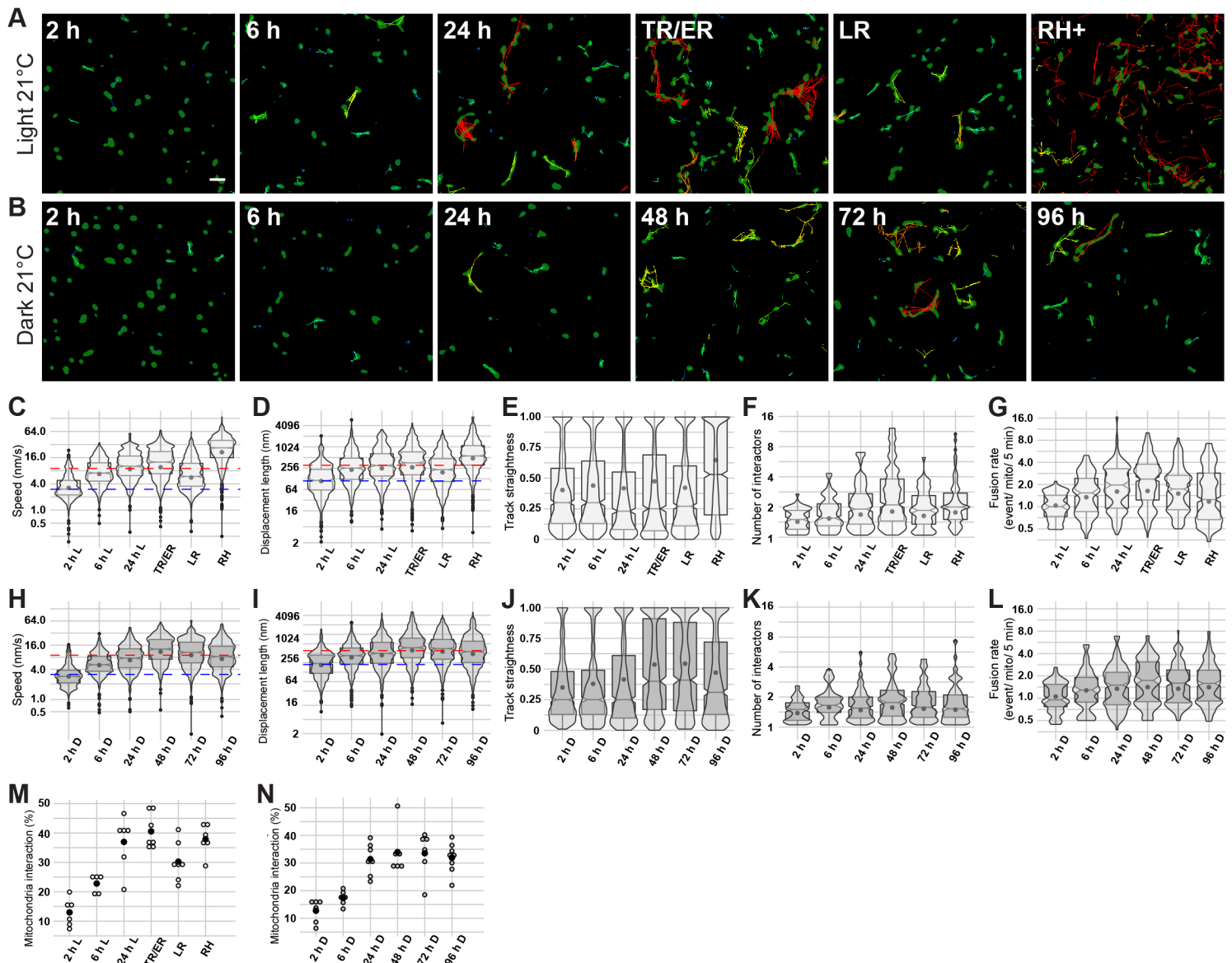
(C) Violin box plots of track displacement length. Plot design as in C.

(D) Violin box plots of track straightness (1 = perfectly straight). Plot design as in C.

(E) Violin box plots of numbers of interactors per track for tracks having at least one interaction between detected mitochondrial objects. Plot design as in C.

(F) Violin box plots of the rate of fusion between mitochondrial objects expressed per mitochondria. Plot design as in C.

(G) Dot plot of the percentage of fusion between mitochondrial objects sharing a track within the 2 min tracking period. Empty circles indicate percentage mitochondrial objects sharing a track in each image stack, while the full circle indicates the mean.

**Supplemental Figure 2**

Reactivation of dynamics of cotyledon mitochondria is delayed without stratification but displays a similar profile

(A), (B) Tracking images of time lapse of cotyledon mitochondria tagged by mito-GFP. Detected mitochondria were colored green and movement was followed over 5 min in order to reconstruct tracks. Seeds were imbibed under light (A) or dark (B) conditions, without stratification. Tracks are rainbow colored according to speed (no movement is purple and movement above 0.032 $\mu\text{m/s}$ is red). Each image is representative of data captured from at least five seeds. Bar = 5 μm . See Supplemental Movie 2. Bars = 5 μm .

(C) to (N) Quantification of the dynamic parameters of tracked mitochondria from seed germinated under light (C to G and M) or dark (H to L and N) conditions, without stratification. The total numbers of tracks analysed per time point range from 659 to 1775. Replication as in A and B.

(C), (H) Violin and box plot of the mean speed recorded per track in nm/s. Box plot whiskers indicate 1.5xIQR and outliers are represented with empty open circle, as described by Tukey. Means are represented by a full circle. The notch corresponds to the mean $\pm 1.58 \times \text{IQR}/\sqrt{n}$. The blue and red lines indicate the median of the mean speed of mitochondria after 48 h of stratification or 2 h after transfer to light/21°C, respectively.

(D), (I) Violin box plots of track displacement length. Plot design as in C.

(E), (J) Violin box plots of track straightness (1= perfectly straight). Plot design as in C.

(F), (K) Violin box plots of numbers of interactors per track for tracks having at least one interaction between detected mitochondrial objects. Plot design as in C.

(G), (L) Violin box plots of the rate of fusion between mitochondrial objects expressed per mitochondria. Plot design as in C.

(M), (N) Dot plot of the percentage of mitochondria sharing a track within the 5 min tracking period. Open circles indicate percentage mitochondrial objects sharing a track in each image stack, while the solid circle indicates the mean. The line represents the Loess regression, while the grey area corresponds to the 95% confidence interval.

One Way Analysis of Variance

Data source: Percentage of mitochondria containing detectable mtDNA nucleoid.

Group Name	N	Missing	Mean	Std Dev	SEM
dry	7	0	0.912	0.0147	0.00556
48h S	7	0	0.908	0.0267	0.0101
TR	7	0	0.744	0.0165	0.00625
RH	7	0	0.674	0.0283	0.0107

Source of Variation	DF	SS	MS	F	P
Between Groups	3	0.301	0.1	200.420	<0.001
Residual	24	0.012	0.000501		
Total	27	0.313			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	$P < 0.050$
dry vs. RH	0.238	19.899	<0.001	Yes
48h S vs. RH	0.235	19.624	<0.001	Yes
dry vs. TR	0.168	14.051	<0.001	Yes
48h S vs. TR	0.165	13.775	<0.001	Yes
TR vs. RH	0.070	5.849	<0.001	Yes
dry vs. 48h S	0.0033	0.276	0.785	No

One Way Analysis of Variance

Data source: Number of nucleiod per mitochondrion

Group Name	N	Missing	Mean	Std Dev	SEM
dry	7	0	1.000	0	0
48h S	7	0	1.002	0.00559	0.00211
TR	7	0	1.367	0.0671	0.0254
RH	7	0	1.000	0	0

Source of Variation	DF	SS	MS	F	P
Between Groups	3	0.703	0.234	206.583	<0.001
Residual	24	0.0272	0.00113		
Total	27	0.73			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
TR vs. RH	0.367	20.365	<0.001	Yes
TR vs. dry	0.367	20.365	<0.001	Yes
TR vs. 48h S	0.365	20.248	<0.001	Yes
48h S vs. RH	0.00211	0.117	0.999	No
48h S vs. dry	0.00211	0.117	0.991	No
dry vs. RH	0	0	1.000	No

Supplemental results

Dynamics of mitochondria in mature green cotyledons.

Mitochondria in relatively mature green cotyledons of 4 day-old seedlings move at much greater speed than those in germinating seeds, and faster still than mitochondria in germinated seedlings at the RH stage, and therefore accurate tracking of each organelle in that tissue necessitated modification of CLSM parameters (faster scan speed) relative to those used for germinating seeds. Images were collected at 1024-pixel resolution, and the PSV 405 nm channel was not used so as to allow an increased frame capture rate of 0.95 fps. The recorded mean speed of mitochondrial movement in 4 days-old cotyledons was over five-times greater, at 360 nm s^{-1} , than that of mitochondria during germination. This greater speed was concomitant with longer displacement values compare to that measured at time points during germination. Mitochondrial dynamics in green cotyledons remains heterogeneous with some mitochondrial displaying very low displacement values ($< 256 \text{ nm}$) due to wiggling motion, which generates short tracks with low straightness values. There is little difference between the numbers of interactors per shared track (cf. Figure 2F and Supplemental Figure 1E), nor in the percentage of mitochondria that interact at least once (cf. Figure 2H and Supplemental Figure 1G), however, the fusion rate in 4 days-old seedlings is approximately 10-times greater than during, or immediately after, germination (cf. Figure 2G and Supplemental Figure 1F, note scales are not identical); although part of this measured increase in the relative rate of fusion may result from the necessary image capture resolution. Nevertheless, a higher fusion rate is an expected outcome of an increase in mitochondrial speed due to the higher likelihood of faster-moving mitochondria encountering a fusion partner, however, the fact that mitochondria in 4 days-old seedlings are typically round to spherical rather than tubular products of repeated fusion events suggests that fusion is rapidly followed by subsequent division to

maintain a fragmented chondriome in mature green cotyledons compared to the uncoupling of fusion from division that occurs during ER/TR stages in germinating seed.

Dynamics with no stratification

Seed were stratified to minimize variation due to variable dormancy between biological replicates thereby helping synchronize germination, and also to enable direct comparison with previous studies (Law et al., 2012). Whilst stratification replicates a natural phenomenon, we wanted to rule out possible artefactual effects of the process on the reactivation of mitochondrial dynamics. Seed were surface sterilized and incubated directly under light/21°C. After 2 h of imbibition, few mitochondria displayed a reactivation of mitochondrial dynamics and instead they behaved like mitochondria under stratification (Supplemental Figure 1A and C, blue = median speed after 48 h stratification). Mitochondrial speed of movement increased during continued imbibition both in the light and dark but it took more than 6 h incubation before the median speed reached that of mitochondria in seed stratified for 48 h and then incubated at light/21°C for 2 h (Supplemental Figure 2C, red line = median at 50 h L). Following 24 h light/21°C, seed were close to germinating (but before testa rupture stage), and mitochondrial dynamics had increased relative to 6 h: 37% of mitochondria were interacting and the fusion rate per mitochondrial had increased substantially (Supplemental Figure 2, Supplemental Movie 2). Mitochondrial fusion and the number of interacting partners were highest at the end of germination (ER/TR stages, Supplemental Figure 1 F, G and O) leading to the formation of perinuclear cage as observed and described at the same developmental stage in stratified seed. Similar to stratified seed, the newly formed cage is a transient structure and a reduction in mitochondrial speed, fusion rate per mitochondrion, number of interacting partners accompanies the fragmentation of the reticular structure as observed with stratified seed (Supplemental Figure 2 cf. Figure 2). During early seedling growth mitochondrial dynamics changes dramatically with mitochondria moving at much greater speeds, following straighter tracks over greater

distances resulting in a reduction in the fusion rate per mitochondrion. Reactivation of dynamics also occurred in the dark although mitochondria were slower to reactivate relative to incubation in the light, showing slower speeds, along less-straight tracks resulting in lower rates of fusion per mitochondrion (Supplemental Figure 2B L & N) and lower numbers of interacting partners early during imbibition (Supplemental Figure 2M) such that 24 h incubation in the dark was required for the dynamics parameters to reach levels similar to those measured after only 6 h in the light. The initially lower dynamics parameters measured in the dark were coincident with a failure to form the perinuclear cage (Supplemental Movie 2). Even after 48 h, when larger values of mitochondrial speed, displacement and track straightness were measured relative to 24 h L or 24 h D these activities were not translated into a greatly increased fusion rate, number of interactors or percentage of interactions (Supplemental Figure 2) such that clusters of mitochondria formed but these did coalesce into the higher-ordered perinuclear cage. Despite an increase in dynamics over the first 48 h in the dark, further incubation up to 96 h D had little effect beyond a slight decrease in fusion rate, number of interactors and percentage interactions. Together these results demonstrate that the reactivation of mitochondrial dynamics occurs similarly in stratified and non-stratified seeds, with stratification acting to reduce the time required for reactivation of mitochondrial dynamics. In addition, it is clear that formation of the mitochondrial cage is tightly linked to the completion of germination and is required to generate the highly dynamic and metabolically active mitochondria of the developing seedling.